

Ser. No. 09/576989
Atty. Docket No. 56039-4356

Listing of the Claims:

1. (currently amended) A polynucleotide comprising a non-naturally occurring HCV subtype 1b sequence that is capable of productive replication in a host cell, or is capable of being transcribed into a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, wherein the HCV sequence comprises, from 5' to 3' on the positive-sense nucleic acid, a functional 5' non-translated region (5' NTR); one or more protein coding regions, including at least one polyprotein coding region that is capable of replicating HCV RNA; and a functional HCV 3' non-translated region (3' NTR), wherein said polynucleotide further comprises an adaptive mutation in the NS5A coding region such that the adaptive mutation results in a change in the NS5A amino acid sequence selected from the group consisting of Ser (1179) to Ile, Arg (1164) to Gly, Ala(1174) to Ser, Ser(1172) to Cys, and Ser(1172) to Pro of SEQ ID NO:3 that confers improved cell culture characteristics to said polynucleotide.

2. (canceled)

3. (previously presented) The polynucleotide of Claim 1, having a transfection efficiency into mammalian cells of greater than 0.01%.

4. (original) The polynucleotide of Claim 3, wherein the transfection efficiency into mammalian cells is greater than 0.1%.

5. (original) The polynucleotide of Claim 3, wherein the transfection efficiency into mammalian cells is greater than 1%.

6. (original) The polynucleotide of Claim 3, wherein the transfection efficiency into mammalian cells is greater than 5%.

Scr. No. 19576989
Atty. Docket No. 56294356

7-8. (canceled)

9. (previously presented) The polynucleotide of Claim 1, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

10-28. (canceled)

29. (previously presented) The polynucleotide of Claim 1, wherein the transfection efficiency into mammalian cells is about 6%.

30-60. (canceled)

61. (original) The polynucleotide of Claim 1, wherein the polynucleotide is double-stranded DNA.

62. (original) A vector comprising the polynucleotide of Claim 61 operably associated with a promoter.

63-68. (canceled)

69. (currently amended) An isolated host cell or a non-human transgenic organism comprising the vector of Claim 62.

70. (currently amended) An isolated host cell or an isolated host cell engrafted in a host organism comprising the polynucleotide of Claim 1, wherein the isolated host cell is a mammalian cell.

Ser. No. 09/576989
Atty. Docket No. 56029-4356

71. (canceled)

72. (currently amended) The isolated host cell of Claim 70 wherein the isolated host cell is a human cell.

73. (currently amended) The isolated host cell of Claim 72 wherein the isolated host cell is a liver cell.

74-85. (canceled)

86 [[87]]. (previously presented) The polynucleotide of Claim 1, further comprising a mutation in the NS3 or NS4B coding region.

87. (New) The polynucleotide of Claim 1, further comprising a mutation in the coding region of NS3 that results in a change in the NS3 amino acid sequence selected from the group consisting of Gln (87) to Arg and Lys (584) to Glu of SEQ ID NO:3.

88. (New) The polynucleotide of Claim 1, further comprising a mutation in the coding region of NS4B that results in a Ser(925) to Gly change in the NS4B amino acid sequence of SEQ ID NO:3.

89. (New) The polynucleotide of Claim 1, further comprising both a mutation in the coding region of NS3 that results in a Lys (584) to Glu change in the NS3 amino acid sequence of SEQ ID NO:3 and a mutation in the coding region of NS4B that results in a Ser(925) to Gly change in the NS4B amino acid sequence of SEQ ID NO:3.

90. (New) A polynucleotide comprising a non-naturally occurring HCV subtype 1b sequence that is capable of productive replication in a host cell, or is capable of being transcribed into a non-naturally occurring HCV sequence that is capable of

Ser. No. 09676989
Atty. Docket No. 56029-4356

productive replication in a host cell, wherein the HCV sequence comprises, from 5' to 3' on the positive-sense nucleic acid, a functional 5' non-translated region (5' NTR); one or more protein coding regions, including at least one polyprotein coding region that is capable of replicating HCV RNA; and a functional HCV 3' non-translated region (3' NTR), wherein said polynucleotide further comprises an adaptive mutation in the NS5A coding region comprising a deletion of the ISDR encoding region corresponding to nucleotides 5345 to 5485 of SEQ ID NO: 6 that confers improved cell culture characteristics to said polynucleotide.

91. (New) The polynucleotide of Claim 90, wherein the polynucleotide is capable of replication in a HeLa cell.

92. (New) The polynucleotide of Claim 90, wherein the HCV is impaired in its ability to cause disease, establish chronic infections, trigger autoimmune responses, and transform cells.

93. (New) The polynucleotide of Claim 90, having a transfection efficiency into mammalian cells of greater than 0.01%.

94. (New) The polynucleotide of Claim 90, wherein the transfection efficiency into mammalian cells is greater than 0.1%.

95. (New) The polynucleotide of Claim 90, wherein the transfection efficiency into mammalian cells is greater than 1%.

96. (New) The polynucleotide of Claim 90, wherein the transfection efficiency into mammalian cells is greater than 5%.

Ser. No. 09/576989
Atty. Docket No. 55019-4356

97. (New) The polynucleotide of Claim 90, wherein the transfection efficiency into mammalian cells is about 6%.

98. (New) The polynucleotide of Claim 90, wherein the polynucleotide is double-stranded DNA.

99. (New) A vector comprising the polynucleotide of Claim 98 operably associated with a promoter.

100. (New) An isolated host cell or a non-human transgenic organism comprising the vector of Claim 99.

101. (New) An isolated host cell or an isolated host cell engrafted in a host organism comprising the polynucleotide of Claim 90, wherein the isolated host cell is a mammalian cell.

102. (New) The isolated host cell of Claim 101 wherein the isolated host cell is a human cell.

103. (New) The isolated host cell of Claim 102 wherein the isolated host cell is a liver cell.

104. (New) The polynucleotide of Claim 90, further comprising a mutation in the NS3 or NS4B coding region.

105. (New) The polynucleotide of Claim 90, further comprising a mutation in the coding region of NS3 that results in a change in the NS3 amino acid sequence selected from the group consisting of Gln (87) to Arg and Lys (584) to Glu of SEQ ID NO:3.

Ser. No. 09/576989
Atty. Docket No. 50029-4356

106. (New) The polynucleotide of Claim 90, further comprising a mutation in the coding region of NS4B that results in a Ser(925) to Gly change in the NS4B amino acid sequence of SEQ ID NO:3.

107. (New) The polynucleotide of Claim 90, further comprising both a mutation in the coding region of NS3 that results in a Lys (584) to Glu change in the NS3 amino acid sequence of SEQ ID NO:3 and a mutation in the coding region of NS4B that results in a Ser(925) to Gly change in the NS4B amino acid sequence of SEQ ID NO:3.